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NOTES

Erysipelas Serum Titration with Sheep Red Blood Cells Passively Sensitized with a Cellular Extract

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We have demonstrated that sheep red blood cells can be passively sensitized with an extract from *Erysipelothrix rhusiopathiae*. Diagnostic differential hemagglutination titrations may then be made with porcine serum for Erysipelas antibody.

The literature is replete with information about *Erysipelothrix rhusiopathiae*, Erysipelas in swine, and various tests for detection of both antigen and antiserum. This work was undertaken in order to augment or replace the Erysipelas Serum-Culture Agglutination (ESCA) Test (3).

The *Erysipelothrix* extract is simple to prepare and a modification of the method of Keogh (2) as described by Anderson (1) was employed to sensitize sheep red blood cells (SRBC) which requires no special mordant or preparation. Sera need not be sterile and results are known within 2 h. The main purpose of this report is to describe the methodology used to obtain the sensitizing cellular extract and describe some of the results obtained.

Two strains of *E. rhusiopathiae*, XA3 and R-9, were used in this work and they were grown in three media: (i) tryptose broth, without thiamine (Difco); (ii) tryptose broth, without thiamine plus 1% fetal calf serum (Difco); (iii) yeast extract, proteose peptone no. 3 broth. The *E. rhusiopathiae* produced from any combination yielded the same amount of sensitizing extract per unit volume of cells. Incubation time was from 24 to 40 h at 37 C.

The extract is prepared as follows. (i) Centrifuge a sufficient amount of harvested material to obtain a cell pack of about 1 ml, wash pack three times in 9 ml, 0.01 M, pH 7.2 phosphate-buffered saline (PBS); (ii) place cell pack (only) into mortar containing 20 ml of acetone at room temperature; (iii) with a pestle, grind the cell mass thoroughly, allow to settle, and repeat five times; allow mix to dry at room temperature (which may be overnight); (iv) add 10 ml of PBS (as above) to the dried contents and stir, grind, or mix until all is in suspension or solution, or both; (v) centrifuge contents until the supernatant fluid is clear or relatively so. If needed, the supernatant fluid may be filtered through a membrane filter (0.45 μm pore size; Millipore Corp.) without loss of sensitizing activity.

This clarified supernatant material contains the active extract and is ready for use. We have found it beneficial to determine the optimum amount of extract needed for sensitizing the blood. Our experiments have shown that about 0.3 ml per unit of blood is usually optimum per unit of SRBC used (one unit of SRBC equals 0.12 ml of packed cells).

The fluid material has been kept in 1-ml samples at −15 and 4 C. Neither has shown any loss of activity during a 2-month period.

All titrations were performed in U-bottom Microtiter plates. The diluent was PBS with 0.2% bovine serum albumin at pH 7.2. In testing, 0.025 ml of the diluent is added dropwise to the wells of a Microtiter plate. Serum is added to the first well using a 0.025 diluter and is serially diluted through 12 wells. Six to 12 wells are used as blank controls. Sensitized SRBC are added at 0.025 ml to each well. The plate is sealed and the cells are thoroughly mixed and then allowed to stand at 4 C for 1 to 2 h. (The test may be incubated at room temperature; however, our experience indicates that about two wells of sensitivity are lost.) The test

is easy to read since hemagglutinating SRBC form full shield patterns.

Positive serum used for testing was Standard Anti-Swine Erysipelas Serum (USDA-ARS-A1Q-Div., Biologics Services, NADL, Ames, Iowa, for Standard Requirement S-22, Lot No. 11), hereafter referred to as Standard Positive. Negative ESCA Standard serum, swine sera of uncertain (the sera tested in this category had been adjudged ESCA negative, but later swine tests caused doubt) ESCA potency and fetal calf serum were examined. It was demonstrated that Standard Positive serum showed absolutely no hemagglutination pattern when titrated using unsensitized SRBC. Any serum showing hemagglutination activity with sensitized SRBC should be routinely tested with unsensitized SRBC to rule out reactions due to anti-sheep cell antibodies. Thus far, 20 sera of questionable ESCA value have been tested. All were negative as measured by the ESCA test and our hemagglutination method. Table 1, as an example, shows the results obtained on testing the serum of two questionable swine. 2-Mercaptoethanol inactivation of antibody in the Standard Positive serum resulted in no decrease of hemagglutination activity. Thus, the technique is applicable for measuring IgA such as found in swine neonate passive antibody.

The ESCA test has been used for 14 years to determine the antibody level in porcine serum.

TABLE 1. *Serum testing of two swine*[a]

Serum tested	Hemagglutination titration
Standard ESCA negative	4
Fetal calf	0
No. 204 unknown	4
No. 210 unknown	4
Standard positive	128
Diluent only	0

[a] We concluded on this basis that the two swine in question were negative and could be used for vaccination studies without antibody interference.

Our work on this new hemagglutination method has, thus far, demonstrated that this new test has an equal ability to determine the antibody level of all sera tested. Further, the new hemagglutination technique gives results in 2 h as compared to 3 days for the ESCA test.

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